REFRACTIVE INDICES OF PROTEINS IN RELATION TO

AMINO ACID COMPOSITION AND SPECIFIC VOLUME

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As an introduction to the use of refractive index as a method for characterizing proteins, factors involved in the refractive index of proteins have been investigated. Previous investigations, summarized by Doty and Geiduschek (1953), indicate the importance of the composition, density or specific volume, charge, and environmental factors in determining the refractive indices of proteins. However, no systematic investigation has been reported which accounts quantitatively for the refractive indices of proteins. The values reported for the refractive indices of amino acids are scattered and fragmentary (Adair and Robinson, 1930; Craig and Schmidt, 1932). Consequently, the refractive indices of the amino acids present in proteins, as well as several peptides and related substances, have been determined in order to calculate the effect of composition on refractive index.

The refractive indices of protein and amino acid solutions were determined by means of a dipping refractometer using a sodium light and also with the Brice and Halwer (1951) differential refractometer. Concentrations of the protein and amino acid solutions were based on the dry weight of an aliquot at 110° and also by moisture determinations.

The amino acids were high grade commercial products. They were further purified by recrystallization from alcohol-water mixtures, with the exception of samples of chromatographically pure amino acids. No difference was found

between the refractive indices of the DL amino acids and the corresponding optical active amino acids; consequently, most of the measurements were made on the DL amino acids. Refractive index measurements were made on amino acid concentrations of 1 to 10% depending on the solubility of the amino acid. No difference in refractive increment was found due to variations in concentration except in the case of glycine solutions. For these the difference could be correlated with variations in specific volume of glycine and were essentially eliminated by applying the appropriate specific volume for a given concentration of glycine. Cystine, tyrosine and aspartic acid are not sufficiently soluble in water for accurate measurements of their refractive indices. The molar refraction of cystine was calculated with the use of the following molar atomic refractions in cc. given by Fajans (1960): C 2.48, H 1.100, O^I 1.525, O^{II} 2.211, N 2.322, S 8.11. It is of interest to note that the calculated molar refractions of the aliphatic amino acids obtained by means of these atomic refractions are in excellent agreement with the observed values.

The molar refractions of tyrosine and aspartic acid were estimated from the total molar refractions of glycyl tyrosine and glycyl aspartate, respectively, by subtracting the refraction due to the glycyl residue. Molar refractions of the amino acids were calculated from the refractive index measurements on amino acid solutions by the Lorentz and Lorenz equations as summarized by Doty and Geiduschek (1955). The molar refractions of the amino acids, as well as the refractivities per gram of their residues, are recorded in Table I. The refractivity per gram residue of amino acid is obtained by subtracting the value of 3.73 (the molar refractivity of water) from the molar refractivity of the amino acid and then dividing the result by the molecular weight of the amino acids. The value of 3.73 for the molar refraction of water is the sum of its atomic fractions, namely, 2H = 2.2, O = 1.525 or 3.73. This value for the molar refraction of water is in essential agreement with the value for water deduced from the molar refraction of glycine and its peptides. The molar refraction of glycine is 16.54, diglycine 29.89, and triglycine 41.33. Thus, by difference, the loss of a molecule of water in making diglycine

TABLE I

Molar Refractions of Amino Acids and the
Calculated Refractions of the Amino Acid Residues
25° Sodium Light

Amino Acid	Molar Refraction of the Amino Acid Observed	Molar Refraction of the Residue (Refraction of Amino Acid - 3.73)	Refraction per gm. Residue (Mol. Ref. Residue)(Mol. Wt. Residue)
	cc.	cc.	cc.
Glycine	16.54 ± .1	12.81	.225
Alanine	20.88 ± .15	17.15	.242
Valine	30.46 ± .13	26.73	.270
Leucine	35.32 ± .15	31.59	.279
Isoleucine	35.60 ± .20	31.87	.282
Serine	22.89 ± .10	19.16	.220
Threonine	27.55 ± .10	23.82	.236
Hydroxyproline	29.57 ± .10	25.84	.229
Proline	27.47 ± .10	23.74	.245
Methionine	38.18 ± .05	34.45	.263
Cystine	56.04	48.58 [a]	.238
Phenylalanine	45.94 ± .15	42.21	.287
Tyrosine	48.07	44.34	.272
Tryptophane	58.97 ± .30	55.24	.297
Histidine	38.35 ± .15	34.62	.253
Arginine	43.20 ± .10	39.47	.253
Lysine	37.83 ± .2	34.10	.266
Aspartic acid	29.79	26.06	.227
Glutamic acid	33.80 ± .15	30.07	.233
Asparagine	29.82 ± .20	26.09	.229
Glutamine	34.10 ± .20	30.37	.237

[a] 2 moles of water subtracted (7.46 cc.).

results in a decrease in refraction of 3.19 and the loss of two molecules of water in making triglycine gives a decrease in refraction of 3.64 per mole of water.

Crystalline ribonuclease and pepsin were obtained from the Armour Laboratories[*] and β-lactoglobulin was prepared from skimmed milk. The mean

[*]It is not implied the USDA recommends the above company or its product to the exclusion of others in the same business.

refractive indices of the proteins were calculated from the refractive indices

measurements on 1 and 2% solutions of the proteins in water by the same method

as was used for the calculation of the refractive indices of the amino acids.

The method used for calculating the refractive index of a protein from

its amino acid composition is essentially the same as that described by Cohn

and Edsall (1943) for calculating the specific volume of a protein from its

amino acid composition, the principal difference being that the refraction per

gram of the residue as given in Table I is used instead of the specific volume

of the residue. The weight per cent of each amino acid residue in the protein

is multiplied by the refraction per gram of its residue to obtain the refrac-

tion due to the amino acid residue in the protein. The summation of the amino

acid residue refractions should give the value of refraction of the residues

in 100 grams of protein. However, the sum of the weight of the residues

seldom equals exactly 100%; consequently, it is necessary to divide the sum

of the weight of the residues into the sum of the refractions of the residues

and multiply by 100 in order to obtain a more accurate calculation of the

refraction of 100 grams of protein. The calculated refractive index of the

protein is then obtained by substituting the value of the refraction of 100

grams of protein and the density of the protein in solution in the Lorentz

and Lorenz equation: $R = \dfrac{n^2 - 1}{n^2 + 2} \times \dfrac{100}{\text{Density}}$, where R is the refraction of 100

grams protein and n is the refractive index of the protein.

In order to obtain agreement between the refractive index of a protein

calculated from its amino acid composition and that determined, it is essential

that the specific volume ($\dfrac{1}{\text{Density}}$) of the protein be accurately known. In the

case of ribonuclease a number of different values have been reported. Rothen's

(1940) value of 0.709 on the ribonuclease prepared by Kunitz is in good agree-

ment with that calculated from its amino acid composition. Using ribonuclease

obtained from the Armour Laboratories, Buzzell and Tanford (1956) found a

value of 0.728 for its specific volume while Harrington and Schellman (1956)

reported a value of 0.692-0.696. These variations in the specific volume are

so large that it was desirable to determine the specific volume of the ribonuclease used. A value of 0.693 for the specific volume of ribonuclease was found which is in excellent agreement with the value of Harrington and Schellman. Its nitrogen content, found to be 16.6%, is also in agreement with the value of 16.8% found by Harrington and Schellman; therefore, the value for the specific volume of ribonuclease of 0.693 was used in calculating its refractive index from the amino acid composition. In the case of pepsin, no value for its specific volume could be located. The specific volume of a 2% pepsin solution was found to be 0.725 in agreement with the value of 0.725 calculated from its amino acid composition. Pedersen's (1936) value of 0.751 was used for the specific volume of β-lactoglobulin.

The results obtained for the refractive indices of several proteins based on the refractive indices of their solutions and the use of the Lorentz and Lorenz equation are compared in Table II with the refractive indices calculated from their amino acid compositions. The results illustrate that the refractive index of a protein is unique and is determined by its composition and specific volume or density.

TABLE II

Specific Volumes and Refractive Indices of Proteins
(25°)

			Refractive Index (589 mμ)	
Protein	Solvent, pH	Specific Volume	Determined	Calculated from Amino Acid Composition[*]
Ribonuclease	Water, pH 4.8	0.693	1.630	1.634[a]
Pepsin	Water, pH 5.0	0.725	1.603	1.609[b]
β-Lactoglobulin	0.1 M NaCl, pH 5.2	0.751	1.594	1.593[c]

[*]Using the amino acid data of: (a) Hirs, Stein and Moore (1954), (b) Blumenfeld and Perlmann (1959), and (c) Gordon et al. (1961).

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